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Thanks!

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# AROMATASE ACTIVITY OF HUMAN GYNECOLOGICAL CARCINOMA CELL LINES

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## ABSTRACT

Human cancer cell lines derived from endometrium (HEC-59, HHUA, Ishikawa, OMC-2), uterine cervix (CAC-1, OMC-4), ovary (2780, 2008, HRA), and breast (MCF-7) were examined for aromatase activity and the effect of estradiol and testosterone on DNA synthesis. Aromatase activity was high (more than 500 fmol/$10^7$ cells/24 hrs) in the cell lines OMC-2 and MCF-7, moderate (100-499 fmol/$10^7$ cells/24 hrs) in OMC-4, HRA, HEC-59, Ishikawa, and CAC-1, and low (less than 100 fmol/$10^7$ cells/24 hrs) in HHUA, A2780, and 2008. A significant stimulation of DNA synthesis (250% increase of [$^3$H]-thymidine uptake) by estradiol ($10^{-9}$ M) was observed in HEC-59, OMC-2, MCF-7, Ishikawa, and HRA. Stimulation of DNA synthesis by estradiol was moderate (115-249%) in OMC-4. Estradiol-dependent increase of DNA synthesis was not observed in HHUA, CAC-1, 2008, and A2780. The cell lines which showed the estradiol dependency in DNA synthesis, namely HEC-59, OMC-2, MCF-7, Ishikawa, and HRA, also presented testosterone dependency. These data suggest that some gynecological carcinomas may possess an aromatase-dependent growth stimulation system. (Jikeikai Med J 1994; 41: 407-15)

Key words: gynecological neoplasms, cell lines, aromatase activity, estrogen dependency, testosterone dependency

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## *INTRODUCTION*

The incidence of endometrial carcinoma has recently been increasing[1]. Obesity, which frequently correlates with a high serum estrogen level[2,3], is a known risk factor for endometrial carcinoma. Many endometrial carcinomas, especially well-differentiated adenocarcinomas, are estrogen-dependent with estrogen receptors. And they frequently respond well to hormonal therapy with progesterone receptors[4,5].

Human breast carcinoma, which is also an estrogen dependent tumor as well as endometrial carcinoma[6,7], has been reported to have aromatase activity[8,9]. Aromatase is an enzyme which converts androgen to estrogen. And aromatase exists in a number of human tissues, including placenta, ovary[10], breast[11,12], adipose tissue[13,14], brain[15,16], and uterine endometrium[17]. Many researchers have reported a positive link between aromatase activity and estrogen-dependent carcinogenesis. The glandular epithelium of the uterine endometrium, where endometrial carcinoma originates, contains both aromatase[18] and estrogen receptors. It is a reasonable assumption that endometrial carcinomas might retain aromatase, and estrogen might be produced in the carcinoma cells by aromatase. This estrogen might bind to estrogen receptors in the carcinoma cells, and growth might be accelerated. There are only few reports on hormone responsiveness of gynecological carcinomas. Clinical trials of some aromatase inhibitors are currently

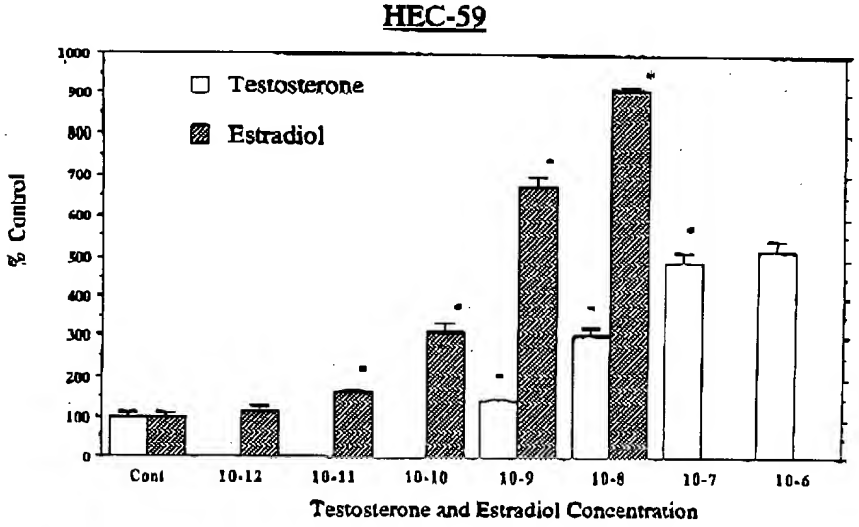


Fig. 1. Response to estradiol and testosterone in DNA synthesis, as measured using the [$^3$H]-thymidine incorporation method, in the HEC-59 (Fig. 1) cells. *$p<0.01$ (Student's *t*-test) comparing to adjacent less concentration of drugs. **$p<0.05$ (Student's *t*-test) comparing to adjacent less concentration of drugs. Each datum was obtained from three independent experiments.

undergoing for breast cancer[17-20], but not yet for endometrial carcinoma.

In this study, we utilized cultured human carcinoma cell lines derived from uterine endometrium, uterine cervix, ovary, and breast to investigate the levels of aromatase activity and effects of sex steroid hormones on DNA synthesis.

## *MATERIALS AND METHODS*

### *1. Cell lines*

The human carcinoma cell lines used in this study were breast carcinoma cell line MCF-7 (American Type Culture Collection, Rockville, MD, U.S.A.), endometrial carcinoma cell lines HHUA[21], Ishikawa[22,23], OMC-2[24], and HEC-59[25], uterine cervical adenocarcinoma cell lines OMC-4[26], CAC-1, and ovarian carcinoma cell lines 2008[27], A2780[28], and HRA[29]. Cells were cultured in Eagle's Minimal Essential Medium (Nissui Co. Ltd. Japan) supplemented with 10% (15% for Ishikawa cell line) fetal bovine serum (FBS), 200 U/ml of penicillin, and 200 $\mu$g/ml of streptomycin, and maintained in a humidified 5% $CO_2$ atmosphere at 37°C.

Steroid free FBS (DCC-FBS) was prepared as follows. 100 ml FBS mixed with 0.25 g activated charcoal (Sigma Chemical Co., St Louis, Mo.) and 0.025 g dextran were stirred at 56°C for 30 min and centrifuged to separate the dextran-coated charcoal pellet

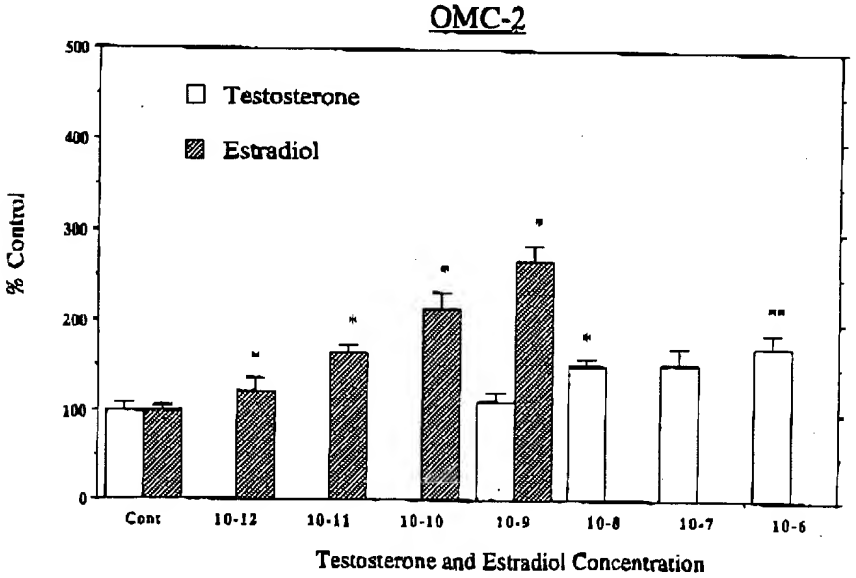


Fig. 2. Response to estradiol and testosterone in DNA synthesis, as measured using the [$^3$H]-thymidine incorporation method, in the OMC-2 cells. *$p<0.01$ (Student's $t$-test) comparing to adjacent less concentration of drugs, **$p<0.05$ (Student's $t$-test) comparing to adjacent less concentration of drugs. Each datum was obtained from three independent experiments.

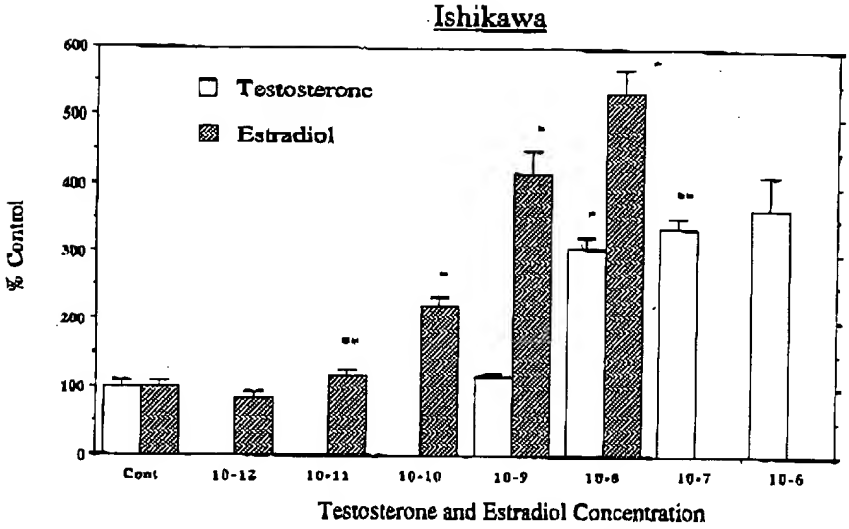


Fig. 3. Response to estradiol and testosterone in DNA synthesis, as measured using the [$^3$H]-thymidine incorporation method, in the Ishikawa cells. *$p<0.01$ (Student's *t*-test) comparing to adjacent less concentration of drugs, **$p<0.05$ (Student's *t*-test) comparing to adjacent less concentration of drugs. Each datum was obtained from three independent experiments.

from the supernatant, which was again subjected to the same treatment at 37°C. The charcoal-stripped serum was filtrated through a 0.2 $\mu$m sterilization unit and stored at −20°C.

*2. Aromatase assay*

For quantitation of aromatase activity, cells were cultured in medium as described above. Twenty four hours prior to aromatase assay, culture medium was changed to the DCC-FBS medium. To start the assay, [1$\beta$-$^3$H] Androstenedione ([1$\beta$-$^3$H] A : $61.05\times 10^6$ dpm/nmol, New England Nuclear, Boston, M.A USA) was added to the culture medium at concentration of 100 nM. After 24 hr, 2 ml of culture medium (from a total of 5 ml) were removed and mixed with 1ml of 20% trichloroacetic acid (TCA) and 1ml of 5% charcoal suspension. The mixture was vortexed several times over a 30 min period, centrifuged for 30 min at 3,000 rpm. and the supernatant was removed and filtered. Aliquots were then measured by liquid scintillation counting.

*3. Effect of hormones on DNA synthesis*

The effects of estradiol and testosterone on DNA synthesis were investigated using the [$^3$H]-thymidine incorporation method, Cells were inoculated into a 96-well dish (Corning Glass Works, Corning, NY, USA) at $10^4$ cells/well in a volume of 0.1 ml of medium

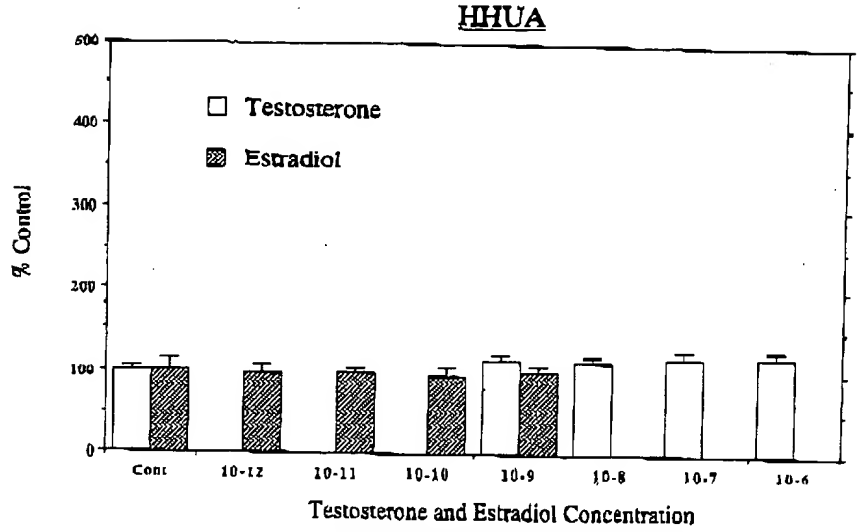


Fig. 4. Response to estradiol and testosterone in DNA synthesis, as measured using the [$^3$H]-thymidine incorporation method, in the HHUA cells. $^*p<0.01$ (Student's t-test) comparing to adjacent less concentration of drugs, $^{**}p<0.05$ (Student's t-test) comparing to adjacent less concentration of drugs. Each datum was obtained from three independent experiments.

Table I. Estrogen and Testosterone Dependency and Aromatase Activities of Cultured Cell Lines Originated from Gynecological Cancer

| Cell line | Origin | Estradiol dependency | Testosterone dependency | Aromatase activity[1] |
|---|---|---|---|---|
| HEC59 | EM Adc. Ca | ## | # | 195.2± 4.5 |
| HHUA | EM Ade. Ca | – | – | 69.2± 2.4 |
| Ishikawa | EM Ade. Ca | ## | # | 249.9±10.7 |
| OMC-2 | EM Ade. Ca | # | + | 514.2± 8.2 |
| CAC-1 | Ut Cerv. Ade. Ca | – | + | 237.4± 7.1 |
| OMC-4 | Ut Cerv. Ade. Ca | + | – | 126.1± 3.9 |
| A2780 | Ov. Ca | – | – | 52.2± 1.1 |
| 2008 | Ov. Ca | – | – | 54.0± 1.3 |
| HRA | Ov. Ca | # | + | 149.8± 3.2 |
| MCF-7 | Breast Ca | # | # | 597.2±10.2 |

EM Adc. Ca; endometrial adenocarcinoma, Ut Cerv. Adc. Ca: uterine cervical adenocarcinoma, Ov. Ca; ovarian cancer.
1; The unit of aromatase activity is fmol/$10^7$ cells/24 hrs.
## indicates more than 500%, # indicates 200-499%, + indicates 115-199%, – indicates less than 115% enhance when $10^{-9}$ M estradiol or $10^{-6}$ M testosterone was added.

containing DCC-FBS, and cultured for 2 days. Estradiol ($10^{-12}$ M-$10^{-9}$ M) or testosterone ($10^{-9}$ M-$10^{-6}$ M) was then added to each well and cultured for an additional 4 days. $^{3}$H-thymidine (0.67 $\mu$Ci, New England Nuclear) was added to the culture medium for 1 hr, the medium was removed. Wells were washed twice with phosphate-buffered saline, once with ethanol, and twice with 5% TCA. Then the remaining non-acid soluble material was solubilized with 1N sodium hydroxide, and aliquots were quantitated by liquid scintillation counting.

## *RESULTS*

### *1. Effects of estradiol and testosterone on DNA synthesis*

Figures 1-4 show [$^{3}$H] thymidine incorporation of endometrial carcinoma cell lines, HEC-59, Ishikawa, OMC-2, and HHUA cells. Estrogen-dependency, testosterone-dependency, and aromatase activity of each cell line are summarized in Table 1. The cell lines HEC-59, MCF-7, OMC-2, and HRA exhibited a high level of responsiveness to estradiol, with more than a 250% increase in DNA synthesis with the addition of estradiol at $10^{-9}$ M. The cell lines OMC-4, and Ishikawa exhibited a 115-249% enhancement of DNA synthesis, and the cell lines HHUA, A2780, 2008, and CAC-1 did not show any response to estradiol. Testosterone at $10^{-6}$ M also enhanced DNA synthesis in the HEC-59 and MCF-7 cell lines by more than 200%, but less than 150% in all of the other cell lines. For all of the cell lines studied, response to testosterone was less than that to estradiol. Testosterone responsiveness correlated well with the level of aromatase activity and estradiol responsiveness, except for the CAC-1 cell lines.

### *2. Aromatase activity*

The MCF-7 breast carcinoma cell line demonstrated the highest level of aromatase activity, 597 fmol/$10^{7}$ cells/24 hr, among the 10 cell lines studied. Among the gynecologic carcinoma cell lines, OMC-2 demonstrated the highest aromatase activity, 514 fmol/$10^{7}$ cells/24 hr, which was 86% of the MCF-7 activity. The cell lines HEC-59, Ishikawa, HRA, CAC-1, and OMC-4 demonstrated activities of 126-321 fmol/$10^{7}$ cells/24 hr, 21-54% of the MCF-7 activity. The remaining cell lines, HHUA, A2780, and 2008 demonstrated very low activity, 34-69 fmol/$10^{7}$ cells/24 hr, which was only 5.7-11.6% of the MCF-7 activity. There was no correlation between the level of aromatase activity and tissue of origin of the cell lines (Table 1).

## *DISCUSSION*

The human breast carcinoma cell line MCF-7 is well characterized in regard to its estrogen responsiveness and the ability to synthesize endogenous estrogen with aromatase[30,31]. Although there have been a significant number of reports concerning this phenomenon in MCF-7 cells, data on the ability of gynecological carcinoma cell lines to

carry out aromatase-mediated estrogen biosynthesis are scant. Currently, it is not clear whether aromatase is involved in the growth stimulation of gynecological carcinoma cell lines. In this report, aromatase activity and the effects of estradiol and testosterone on DNA synthesis were verified in gynecological carcinoma cell lines.

Although the HEC-59 and Ishikawa cell lines showed the strongest response to estradiol and testosterone in DNA synthesis, the aromatase activity level was moderate (195.2 and 249.9 fmol/$10^7$ cells/24 hrs. respectively) and was lower than that of the MCF-7 and OMC-2 cell lines (597.2 and 514.2 fmol/$10^7$ cells/24 hrs. respectively). On the other hand, response to estradiol in DNA synthesis was moderate in the OMC-2 cell line, aromatase activity was high.

The cell lines with low aromatase activity, namely HHUA, A2780 and 2008 (69.2, 52.2 and 34.0 fmol/$10^7$ cells/24 hrs. respectively), did not demonstrate estrogen responsiveness. These data indicate that high aromatase activity correlates with a greater response to estradiol and testosterone in DNA synthesis.

Nine cell lines originating from gynecological carcinomas were investigated in this study, and the HEC-59, OMC-2, and HRA cell lines demonstrated high hormone responsiveness. The MCF-7 breast carcinoma cell line is widely used for the investigation of aromatase activity and the effects of aromatase inhibitors on cell growth.

It is difficult to estimate the level of aromatase activity which is required for cell growth, but the HEC-59, OMC-2, and HRA cell lines may possess an adequate amount of aromatase to convert androgen to estrogen for cell growth. Some aromatase inhibitors might therefore be effective in inhibiting the growth of these cell lines.

It is reported that estrogen biosynthesis is inhibited by aromatase inhibitors *in vivo* and *in vitro*[18,31-33], and anti-tumor effect of aromatase inhibitors has also been reported[18,32,34]. Nowadays, aromatase inhibitors are on clinical use for breast cancer[18,32,34]. The principal site of estrogen biosynthesis in postmenopausal women is adipose tissue[13,35,36], the most frequent age for gynecological cancer is also postmenopause. Aromatase inhibitors may be useful for gynecological cancer therapy not only by inhibiting aromatase activity of the cancer cells, but also by decreasing the aromatase activity in adipose tissue, resulting in a decreased serum estrogen level.

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